Antibacterial effect of composite resins containing bioactive glass-ursolic acid on the biofilm of Streptococcus mutans

Bona Kim

The Graduate School

Yonsei University

Department of Dental Science

Antibacterial effect of composite resins containing bioactive glass-ursolic acid on the biofilm of Streptococcus mutans

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Bona Kim

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This certifies that the Masters Thesis of

Bona Kim is approved.

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Jeong-Won Park

_____

Byoungduck Roh

_____

Sung-Ho Park

The Graduate School

Yonsei University

December 2012

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또한, 바쁘신 와중에도 실험 과정 내내 많은 도움을 주신 서울대 교정학교실 안석준 교수님, 박소윤 선생님, 조은정 선생님께도 감사의 말을 전하고 싶습니다

마지막으로 늘 곁에서 응원해주며 한결 같은 사랑을 주시는 존경하는 부모님과 바쁜 와중에도 항상 제게 힘이 되어주는 사랑하는 동생들, 다슬이와 예슬이에게 그 동안 못다한 감사와 사랑을 전합니다.

2012년 12월

김 보 나

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Abstract

Antibacterial effect of composite resins containing bioactive glass-ursolic acid on the biofilm of Streptococcus mutans

Bona Kim, D.D.S.

Department of Dental Science, Graduate School, Yonsei University

(Directed by Prof. Jeong-won Park, D.D.S., M.S., Ph.D.)

1. Objective

The aim of this study was to figure out which methods are more effective for inhibiting the *S. mutans* biofilm formation when we add the bioactive glass and/or ursolic acid in the experimental composites.

2. Materials and methods

Four antibacterial composites (71% Sr glass filler, 29% 50:50 bis-GMA:TEGDMA) containing bioactive glass (BAG) (62mol% SiO_2, 31mol% CaO, 4 mol% P_2O_5, and 3 mol% F) filler, BAG filler coated with ursolic acid (UA BAG), ursolic acid was added to the resin matrix (BAG + UA monomer), ursolic acid was added both BAG filler and resin matrix

(UA BAG + UA Monomer) were developed. Conventional composite (10%wt OX50 silica nanofiller instead of BAG)) were used as control groups. For biofilm assay, *S. mutans* was incubated for 24 hours with each composite resin disk specimen in a biofilm medium with either glucose or sucrose in the presence or absence of a salivary coating. The adherent bacteria were quantified after sonication of the specimen by counting the colony forming units of viable bacteria. For the growth inhibition test, the crescent-shape resin specimens with either saliva coating or non-coating were placed on a polystyrene well cluster, and *S. mutans* suspension in trypton-vitamin medium with either glucose or sucrose was inoculated. The OD_{600} values were recorded for 24 hours. Two-way ANOVA, with Bonferroni correction, was used for analysis (α=0.05).

3. Result

All composites containing BAG and UA (BAG, UA BAG, BAG + UA Monomer, UA BAG + UA Monomer) significantly reduced the amount of biofilm formation of *S. mutans* regardless of the carbohydrate source and the salivary condition.

When glucose was given as a carbohydrate source, the CFU values significantly decreased in the threeBAG added UA groups (UA BAG, BAG + UA Monomer, UA BAG + UA Monomer) than BAG group. Among them, significantly lower CFU value in BAG+ UA Monomer group. There was no significant differences between UA BAG group and UA BAG + UA Monomer group.

On the contrary, when sucrose was given as a carbohydrate source, there were no significant differences among BAG, UA BAG, BAG + UA Monomer, and UA BAG + UA Monomer groups.

The bacterial growth curve results did not show any difference contrary to those of biofilm assay.

4. Conclusion

Experimental composites containing BAG showed a significant reduction of the biofilm formation by S. *mutans*in any salivary treatment and carbohydrate sources for less than 24 hours. When glucose was given as a carbohydrate source, treatment with ursolic acid show additional effects of decreasing biofilm formation. Especially among them, composites containing BAG fillers and UA in the matrix group showed more significant antibacterial effect compare to other groups. On the contrary, when sucrose was given, treatment with ursolic acid did not show any additional effects of decreasing biofilm formation.

The bacterial growth curve results did not show any difference contrary to those of biofilm assay.

Key words : bioactive glass, ursolic acid, antibacterial composite resin, bioflm,

Streptococcus mutans

Antibacterial effect of composite resins containing bioactive glass-ursolic acid on the biofilm of Streptococcus mutans

Directed by Prof. Jeong-won Park

The Graduate School, Yonsei University

Department of Dental Science

Bona Kim

I. Introduction

Dental resin composites are widely used in restorative dentistry for treatment of dental caries (Mjor et al., 1999). In the past few decades, the mechanical properties, wear resistance, physical and bonding properties have

been greatly improved. However, secondary caries is the most frequent failure in composite restoration (Opdam et al., 2007). To overcome this problem, a number of studies have tried to develop antibacterial composite resins using various methods, including addition of antibiotic compositions, such as chlorhexidine diacetate (CHXA), sliver nanoparticles, monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB), quaternary ammonium polyethylenimine (PEI) nanoparticles, and alkylated ammonium chloride derivatives(Cheng et al., 2012; Hiraishi et al., 2008; Imazato et al., 1995). Generally, the site incorporated antibacterial agents is resin matrix or filler. The addition of antibacterial agents into resin matrix, such as CHXA, sliver nanoparticles, monomer 12-MDPB was developed (Imazato et al., 1997; Leung et al., 2005). Several caries-prevention fillers, such as dicalcium phosphate anhydrous (DCPA, $CaHPO_4$)-whiskers, tetracalcium phosphate (TTCP, $Ca_4(PO_4)_2O$)-whiskers, calcium fluoride (CaF_2), and polymer-kaolinite, were attempted to develop the antibacterial composite resin (Xu et al., 2008; Xu et al., 2007, 2009). However, experimental antibacterial composite resins had several limitations; short-term effectiveness, decreased physical property, possible toxicity, or poor color stability (Fan et al., 2011; Kohnen and Jansen, 1995; Nohr and Macdonald, 1994). Therefore there are no commercially successful antibacterial composite resins. Those limitations are related to the characteristics and the size of the antibacterial materials, and the site of incorporation-resin matrix or filler components.

Bioactive glasses (BAG) are used for hard tissue replacement. Bioactive glasses are biocompatible and stimulate the regeneration of bone(Ohtsuki et

al., 1991). This material is used in dental implants, bone cement, bone and periodontal fillers. Also, BAGs form a layer of carbonated apatite on their surface, when placed on the dentin and enamel. Therefore BAGs are used in restorative dentistry for the remineralization of enamel and dentin. (Efflandt et al., 2002). BAGs have biomimetic properties when immersed in body fluids, leading to the formation of tooth-like hydroxyapatite (Forsback et al., 2004). BAGs can reduce tubule fluid flow by occluding of dentinal tubule, ultimately resulting in reduced dentinal hypersensitivity (Mitchell et al., 2011). They also reduce with spot lesion formation (Brown et al., 2011). In addition, BAG powders shows the complete inhibition of the growth of *Streptococcus mutans* after only 2 hours of contact with the BAGs powder(Catauro et al., 2004). Furthermore, these materials are relatively inexpensive and are easy to synthesize (Mitchell et al., 2011).

Ursolic acid is triterpenoid compounds that exist widely in food, medicinal herbs and other plants. Ursolic acid is well known natural as derivatives having anti-inflammatory, anti-tumor, and anti-microbial effects(Liu, 2005). Ursolic acids inhibit the growth and survival of many bacteria, particularly gram-positive species, including pathogenic ones (Kurek et al., 2010). Ursolic acid was found to suppress the bacterial growth of *S. mutan*s when they were added to the matrix of commercial nanofilled composite (Filtek Z350, 3M ESPE, St Paul, MN, U.S.A.) in previous studies (Kim, 2011). Due to hydrophobic nature of triterpenoids, it might induce them to blend properly with the composite resins and not easily eluted into the saliva, thus long-lasting antibacterial effects could be expected. In addition, ursolic acid is

relatively non-toxic and had no side effect(Liu, 1995). Therefore, ursolic acid was also assumed to be very useful supplements of antibacterial composite resins.

As mentioned above, ursolic acid was found to suppress the bacterial growth of *S. mutans* when they were added to the matrix of commercial nanofilled composite (Filtek Z350, 3M ESPE, St Paul, MN, U.S.A.) in the previous studies(S. Kim, 2011). The site of incorporation was resin matrix. However, BAGs treated with ursolic acid did not show the additional effects of decreasing biofilm formation in the other previous study(Y. Kim, 2012). In this study, the site of incorporation was filler. The site of incorporation appears to be a key determinant of the function of antibacterial agents whether into filler or into resin matrix.

Generally, Ebi et al reported that the addition of antibacterial agents into filler components appears a more reliable approach than the addition into resin matrix (Ebi et al., 2001). However, reliable site of incorporation is controversial.

The aim of this study was to figure out which methods are more effective for inhibiting the *S. mutans* biofilm formation when we add the bioactive glass and/or ursolic acid in the experimental composites.

The null hypothesis was that there was no difference for adding methods on the inhibition of biofilm formation.

II. Materials and Methods

1. Preparation of experimental composite resin

Four antibacterial composites containing bioactive glass (BAG)(62 mol% SiO_2, 31 mol% CaO, 4 mol% P_2O_5, and 3 mol% F) filler were developed. Filler and matrix compositions of experimental groups are as displayed in Table 1. Ursolic acid (UA) is the most representative triterpenoids, were coated to BAG fillers (UA BAG groups), ursolic acid was added to the resin matrix (BAG + UA Monomer groups), ursolic acid was added both BAG filler and resin matrix (UA BAG + UA Monomer groups) were developed. Conventional composite (10%wt OX50 silica nanofiller instead of BAG) were used as control groups. BAGs were synthesized by sol-gel methods, ball milled, sieved, and micronized (Sturtevant, Hanover, MA, USA). Average particle size ranged from 0.04 to 3.0 μm, as determined by laser particle size measurements (Beckman Coulter LS13 320, Brea, CA, USA) (Brown et al., 2011).

Coating of ursolic acid to BAGs was conducted using vacuum methods. Ursolic acid (U6753, Sigma Aldrich, St. Louis, MO, U.S.A.) dissolved in 70% ethanol was mixed with BAGs. Then, the solvents were evaporated under negative pressure in vacuum condition, and complete evaporation of solvents was confirmed by comparing the weight of before and after the treatment. The BAG fillers, the BAG fillers treated with ursolic acid were incorporated into the matrix of same composition (BAG, UA BAG groups).

To incorporate the ursolic acid into resin matrix, the ursolic acid was mixed with TEGDMA and stirred thoroughly with magnetic stirrer. Afterwards, BisGMA: UDMA was added as 50:50 ratio and prepare the resin matrix for the composite (BAG + UA Monomer groups).

In the same manner as above, ursolic acid was added both BAG filler and resin matrix (UA BAG + UA Monomer groups).

Table 1. Filler and matrix compositions of experimental groups

	Filler (%)				Matrix (%)
	Glass	OX50	BAG	UA	
Control	61	10	0	0	29
BAG	61	0	10	0	29
UA BAG	61	0	9.5	0.5	29
BAG + UA Monomer	61	0	10	0	29(0.5 UA)
UA BAG + UA Monomer	61	0	9.75	0.25	29(0.25 UA)

The composition of matrix used in this study was 49.38% of Bis-GMA, 49.38% of TEGDMA, 0.40% of CQ, 0.80% of EDMAB, and 0.05% of MEHQ.

Control : silica nanofillers BAG: bioactive glass

UA : ursolic acid

Bis-GMA: bisphenol A diglycidyl methacrylate

TEGDMA: triethylene glycol dimethacrylate

CQ: camphorquinone

EDMAB: amine-ethyl-4-dimethylaminobenzoate

MEHQ: monoethyl ether hydroquinone

UA BAG group : Ursolic acid was coated to BAG fillers

BAG + UA Monomer group : Ursolic acid was dissolved in resin matrix

UA BAG + UA Monomer group : Ursolic acid was added both BAG filler and resin matrix

2. Biofilm assay and growth inhibition test

Two methods, biofilm assay and growth inhibition test, were used for evaluating the antibacterial effects of experimental composite resin.

2.1. Assignment of the experimental groups

Four experimental composite resins with bioactive glass (BAG, UA BAG, BAG+UA Monomer, UA BAG+ UA Monomer), control group were compared. For different saliva treatment, the composite resin specimens were submerged in either phosphate buffered saline (PBS, pH = 7.2 : non-coating group) or unstimulated whole saliva (UWS: saliva coating group) and placed on a rocking incubator for 2 hours. For the nutrient source provided for bacterial growth, either glucose or sucrose was added to the medium. The combinations of all these variables are displayed in Figure 1.

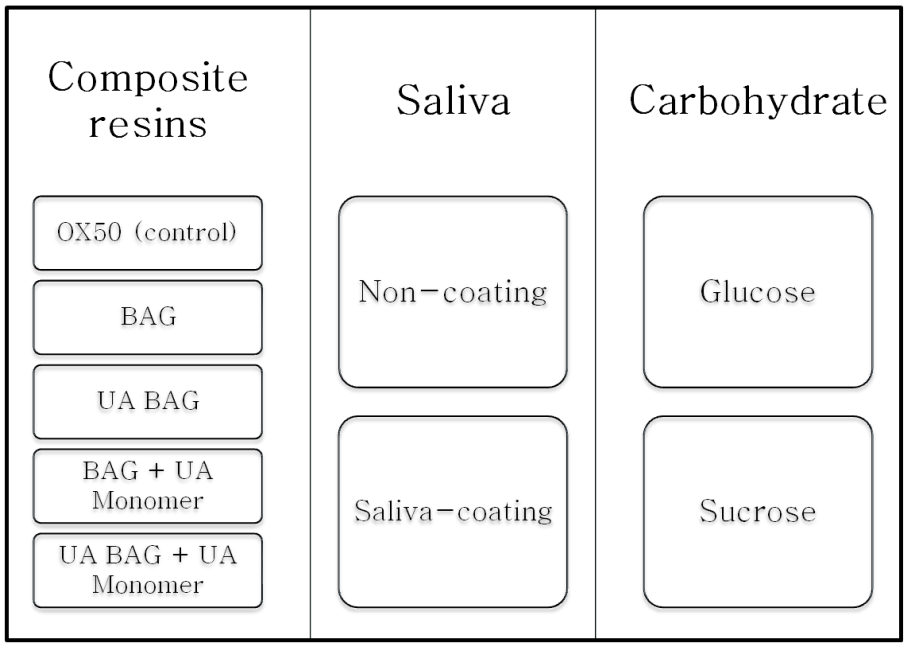

Fig. 1. Assignment of the experimental groups.

2.2. Preparation of *Streptococcus mutans* and saliva

Streptococcus mutans (UA159) was grown in the brain heart infusion (BHI) agar plate. A colony of *S. mutans* was transferred to a BHI broth and the broth was incubated overnight. The cultures were then re-suspended to the BHI broth in 1:20 ratio and incubated again until it reached the exponential phase. The optical density at 600 nm (OD_{600}) was measured, and the broth was used when the OD_{600} reached 0.5 (approximately 6.5×10^7 CFU per mL).

UWS was collected from healthy volunteers who had no acute dental caries or periodontal diseases. The saliva sample was routinely collected between 9:00 to 11:00 A.M. to minimize the effects of diurnal variability on salivary composition (Hardt et al., 2005). The saliva sample was centrifuged at 3,500 x g for 10 minutes to remove any cellular debris. The resulting supernatant was then filter-sterilized through a Stericup & Streitop (Millipore, Billerica, MA, USA), and stored in 4°C before use.

2.3. Biofilm assay

To obtain uniform shape and size of the experimental composite resins, Teflon mold (5 mm in diameter, 2 mm in height) were placed between two glass slides on both sides, and light cured for 40 seconds. Since the biofilm had been formed at the upper surface, extra attention was required not to turn the specimen upside down. Thus the bottom side was marked with an oil-based pen. The upper surfaces of specimens were polished with 800-grit SiC paper (Deerfos, Inchon, Korea), 1200-grit SiC paper(Deerfos), 1500-grit SiC paper(Deerfos) in order. After polishing, they were sonicated for 15

minutes in distilled water to remove debris on the surfaces, and sterilized by EO gas. The surface roughness was measured (mean Ra = 1.54 μm) confirmed that there was no significant differences in each experimental groups.

The sterilized resin disks were transferred to a polystyrene 24-well (flatbottom) cell culture clusters (Corning Inc. Corning, NY, USA). The prepared *S.mutans* suspension was diluted to BHI broth which was kept warm in the incubator. The medium contained 20 mM of either glucose or sucrose as a carbohydrate source. The composite resin disks which were treated with either UWS or PBS were inoculated with those medium containing 1:100 dilution of *S. mutans* suspension. Biofilms were allowed to form at 37° C in a 5% CO_2 for 24 hours.

Afterwards, the disks were washed twice with 2 mL of sterile PBS to remove planktonic and loosely bound cells. The specimen was then placed in a conical tube with 3 ml of PBS and sonicated using 30 seconds pulse at 20 W four times with simultaneous cooling by placing the tube in the ice box. The disrupted biofilm suspension was serially diluted, plated in duplicate on BHI agar, and incubated at 37° C in a 5% CO_2 atmosphere for 48 hours. The plating was carried out by automatic sample plater (easySpiral®, Interscience, Saint Nom, France). The accuracy of dilution and plating by easySpiral® apparatus was confirmed by previous study (Kim, 2011). After 48 hours, colony forming units (CFUs) were counted visually, scaled by dilution factors.

If the CFU values between duplicates differed more than 20%, the data was discarded. For statistical reason, all data acquired on the same day were also

discarded, too. The flow chart of procedures was shown in Figure 2.

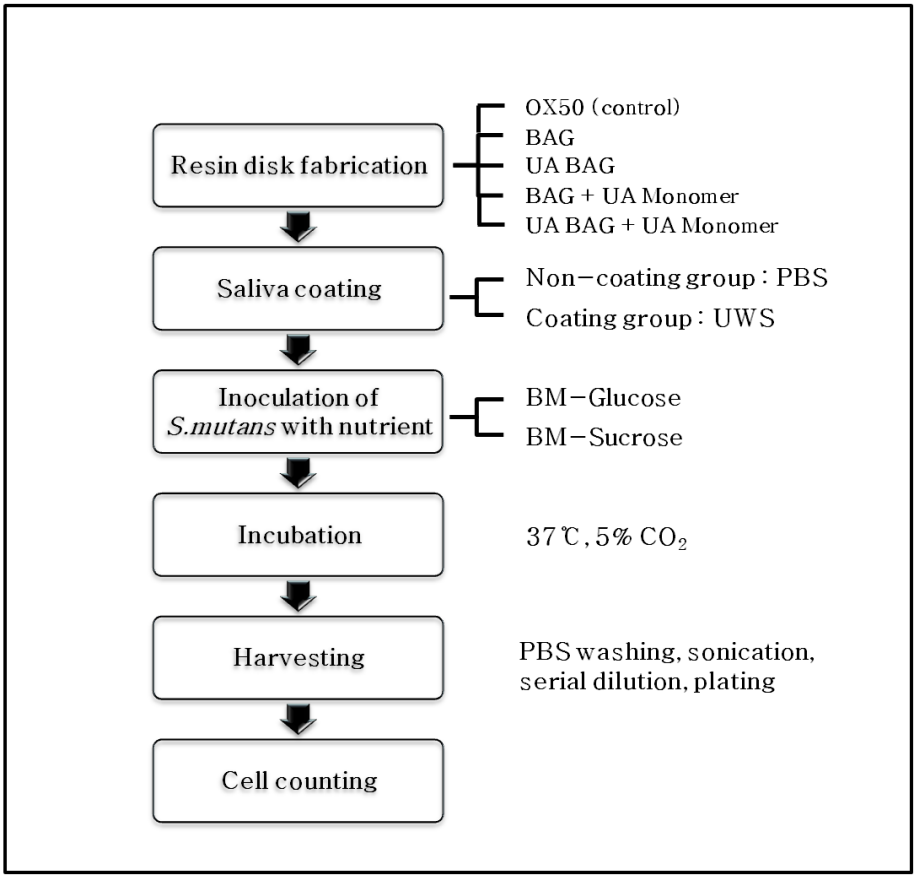

Fig. 2. Procedures of biofilm assay.

PBS : phosphate-buffered saline, UWS : unstimulated whole saliva

BM : biofilm medium

2.4. Growth inhibition test

Growth inhibition test is based on determination of bacterial growth in a polystyrene 96-well (flat-bottom) cell culture clusters (Corning Inc.Corning, NY, USA)(Slutsky et al., 2007). The outgrowth kinetics in each well was recorded continuously by measuring the optical density at 600nm every 30 minutes for 24 hours automatically. An appropriate amount of the

experimental resin was prepared using metal mold. After the upper surfaces of specimens were polished with with 800-grit SiC paper (Deerfos, Inchon, Korea), 1200-grit SiC paper(Deerfos), 1500-grit SiC paper(Deerfos) in order, they were sonicated for 15 min in distilled water to remove debris on the surfaces. The specimens were attached to a crescent-shape at the bottom of the well and light-cured. For each experimental group, 4 specimens were fabricated. The plate was sterilized by EO gas.

200 μL of either PBS or UWS was placed on the specimen for 2 hours. Trypton-vitamin (TV) medium supplemented with 0.5% glucose or sucrose was added with 1:100 dilutions of *S.mutans* broth. TV medium (either with glucose or sucrose) with *S.mutans* was placed in a well for positive control. In each well, sterile mineral oil (50 μL per well) was placed at the top of the TV medium in order to maintain anaerobic condition and prevent the evaporation. The plate was placed in Infinite® F200 pro (TECAN, Salzburg, Austria). The kinetics of outgrowth in each well was monitored at 600nm at 37° C and recorded every 30 minutes for 24 hours using the spectrophotometer. The data analysis was done using Magellan 7 software (TECAN, Salzburg, Austria). If a well was dehydrated due to insufficient protection, a data from the well was discarded. The procedure was performed 3 times. The schematic description of growth inhibition test is displayed in Figure 3.

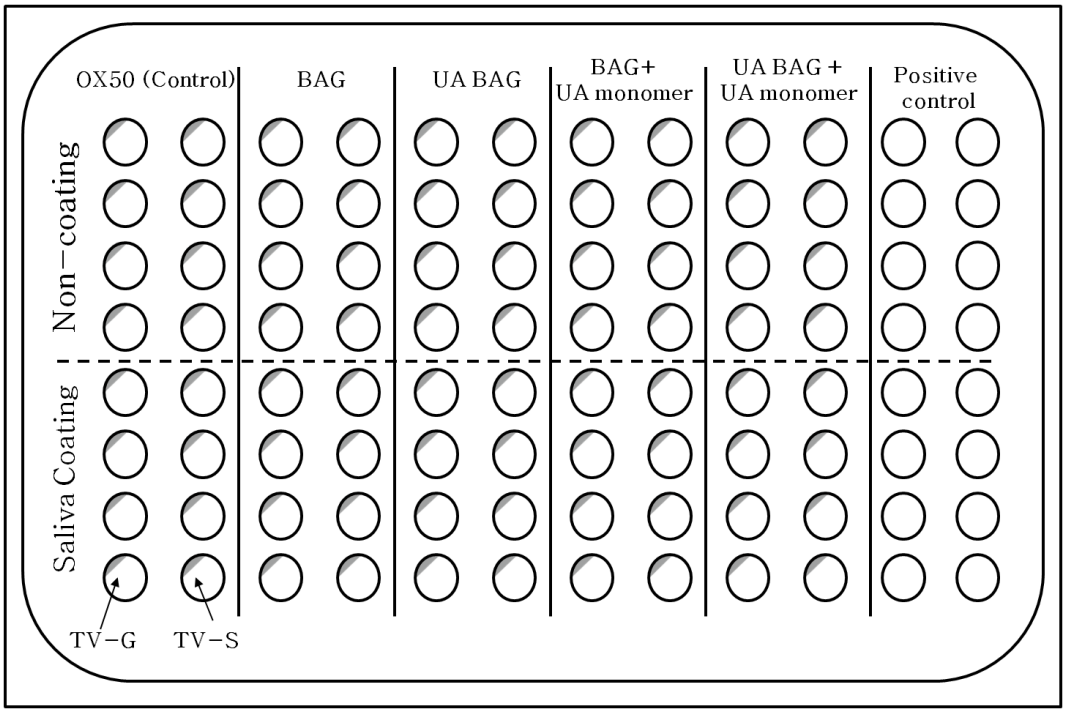

Fig. 3. Schematic description of growth inhibition test.

TV-G : Trypton-vitamin(TV) medium supplemented with 0.5% glucose.

TV-S : Trypton-vitamin(TV) medium supplemented with 0.5% sucrose.

3. Statistical analysis

Surface roughness was compared using Kruskal-Wallis test. Two-way ANOVA followed by the Bonferroni correction were used for bioflim assay to analyze statistical significance. Statistical analyses were performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, U.S.A.). The adjusted P value less than 0.05 was considered to be statistically significant.

III. Result

1. Biofilm assay

The two-way ANOVA demonstrated that there was no interaction effect in sucrose groups. In contrast, there was an interaction effect between the type of composite resins and salivary treatment when glucose was given as a carbohydrate source. In sucrose groups, the CFU scores of S.*mutans* were significantly influenced by both the type of composite resins and salivary treatment.

1.1. The influence of the type of composite resins

As depicted in Fig. 4, the CFU values significantly decreased in the four BAG composite resins than in the control composite regardless of the carbohydrate source and saliva condition.

When glucose was given as a carbohydrate source, the CFU values significantly decreased in the three BAG added UA groups (UA BAG, BAG + UA Monomer, UA BAG + UA Monomer) than BAG group. Among them, significantly lower CFU value in BAG+ UA Monomer group. There was no significant differences between UA BAG group and UA BAG + UA Monomer group (Fig.4(A)).

On the contrary, when sucrose was given as a carbohydrate source, there were no significant differences among BAG, UA BAG, BAG + UA Monomer, and UA BAG + UA Monomer groups (Fig.4(B)).

1.2. The influence of the saliva pretreatment

When glucose was given, significantly lower CFU values in the saliva coating group were found. On the contrary, when sucrose was given, significantly increased CFU values in the saliva coating group were seen.

Table 2. Biofilm formation by *S.mutans* on various experimental groups in the presence of glucose for 24 hours. The amount of bacteria were expressed as CFU/ml

Nutrient	Saliva	Group	Mean	SD
Glucose	Non-coating	Control	3.45×10^6	4.88×10^5
		BAG	2.08×10^6	5.51×10^5
		UA BAG	1.17×10^6	1.34×10^5
		BAG + UA Monomer	7.20×10^5	1.56×10^5
		UA BAG + UA Monomer	1.15×10^6	9.40×10^4
	Coating	Control	1.00×10^6	2.00×10^5
		BAG	7.00×10^5	1.00×10^5
		UA BAG	5.00×10^5	9.00×10^4
		BAG + UA Monomer	3.00×10^5	6.00×10^4
		UA BAG + UA Monomer	4.00×10^5	8.00×10^4

Significance*

Non-coating > Saliva coating

Control> BAG > UA BAG, UA BAG + UA Monomer > BAG + UA Monomer

*Multiple comparisons were performed by *t* tests using the Bonferroni correction at a significant level of $p<0.05$.

Table 3. Biofilm formation by *S.mutans* on various experimental groups in the presence of sucrose for 24 hours. The amount of bacteria were expressed as CFU/ml

Nutrient	Saliva	Group	Mean	SD
Sucrose	Non-coating	Control	1.80×10^6	8.45×10^5
		BAG	9.56×10^5	4.04×10^5
		UA BAG	8.03×10^5	3.32×10^5
		BAG + UA Monomer	5.21×10^5	2.83×10^5
		UA BAG + UA Monomer	8.03×10^5	3.46×10^5
	Coating	Control	3.00×10^6	1.00×10^6
		BAG	2.00×10^6	8.00×10^5
		UA BAG	1.00×10^6	6.00×10^5
		BAG + UA Monomer	8.00×10^5	4.00×10^5
		UA BAG + UA Monomer	1.00×10^6	7.00×10^5

Significance*

Non-coating < Saliva coating

Control > BAG , UA BAG, UA BAG + UA Monomer, BAG + UA Monomer

*Multiple comparisons were performed by *t* tests using the Bonferroni correction at a significant level of $p<0.05$.

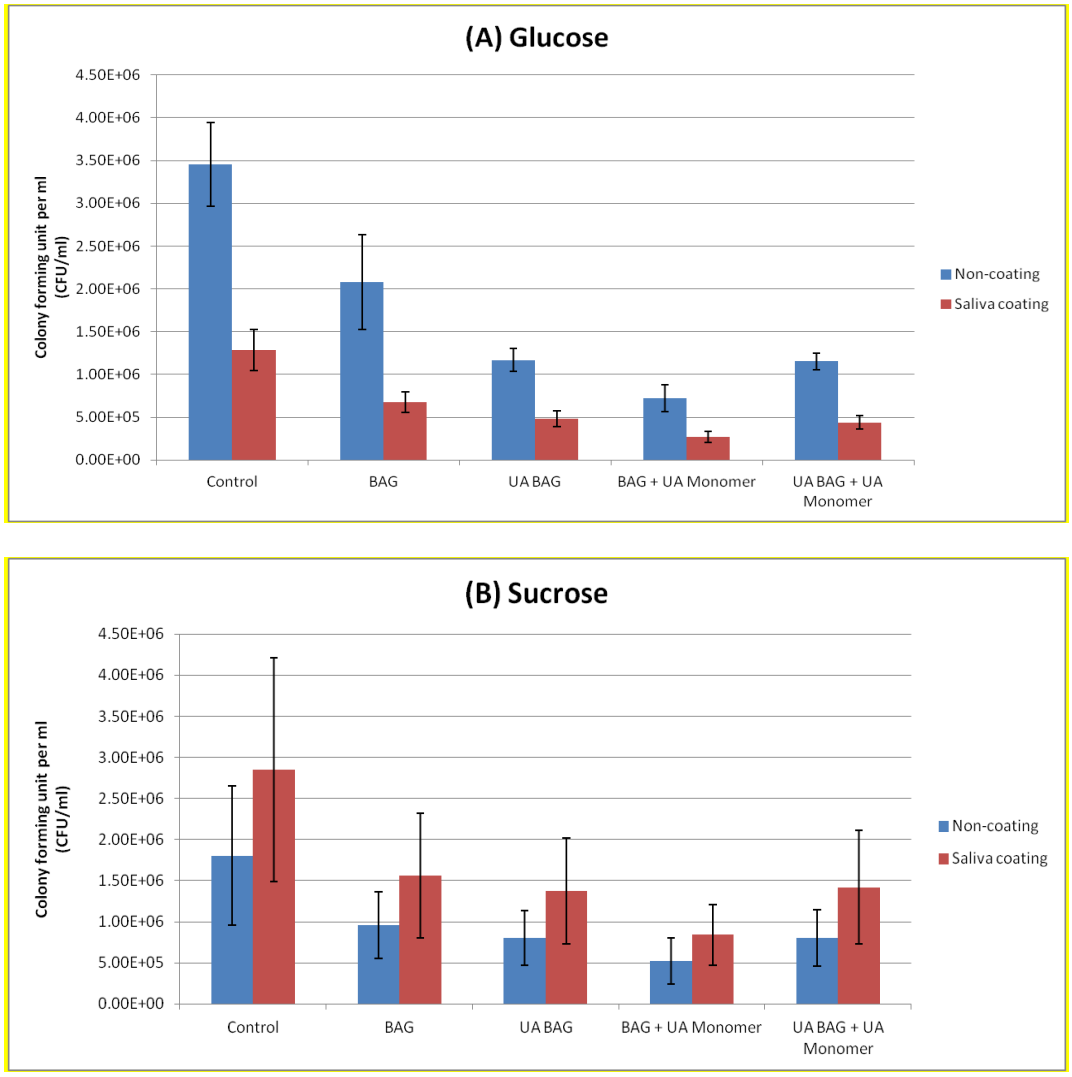

Fig. 4. Biofilm formation by *s.mutans* on various experimental composite resins in in BM glucose(A) and BM sucrose(B).

2. Growth inhibition test

This result of bacterial growth curve was contrary to results of biofilm assay. In all groups, antibacterial effect was not shown. In all types of experimental composite resins, the bacteria entered stationary phase at an optical density in both TV-glucose and TV-sucrose medium (Fig. 5).

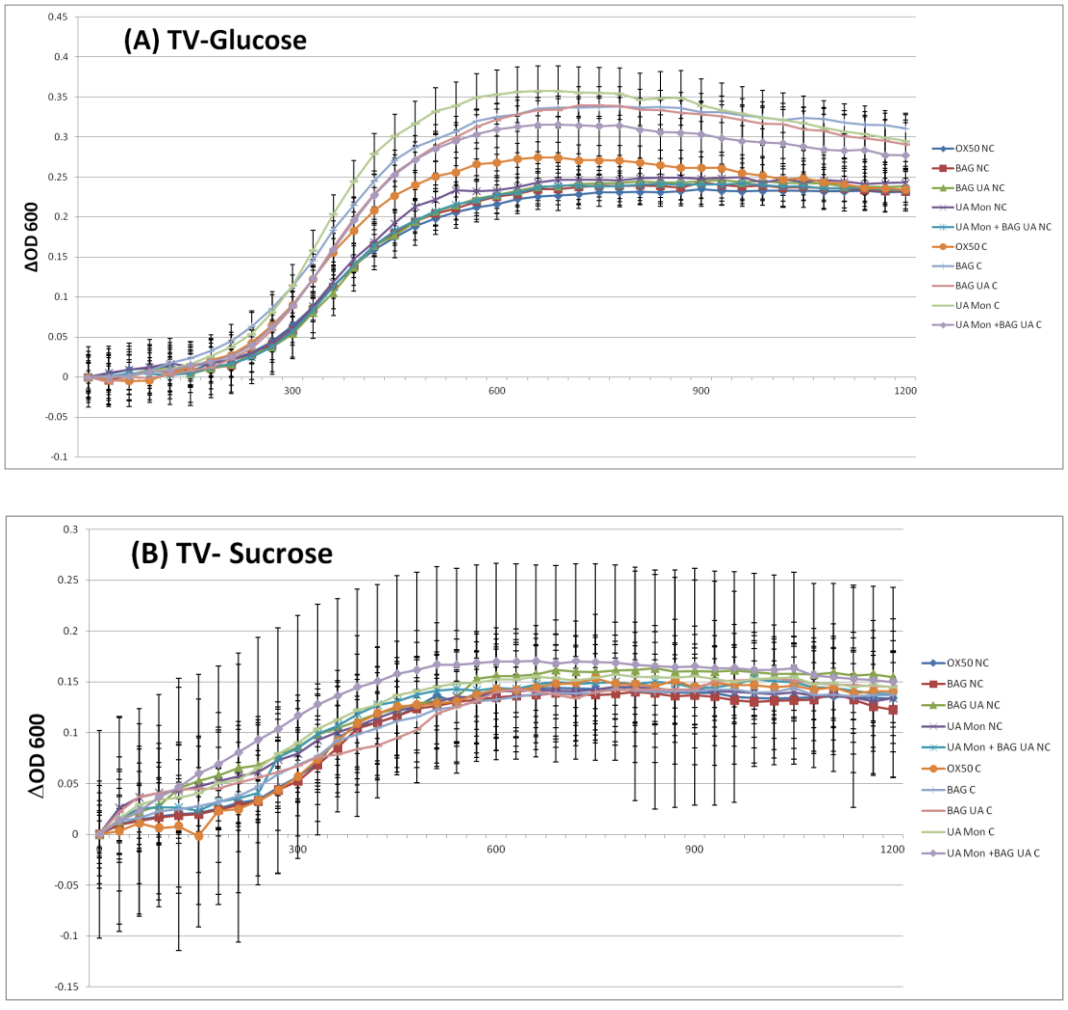

Fig. 5. Growth curve of S.mutans in TV-glucose(A) or TV-sucrose(B) media on various experimental composite resins, Data are expressed as means ± standard error of OD_{600}. NC : non-coating, C: saliva coating

IV. Discussion

This study assessed the *Streptococcus mutans* biofilm formation on the experimental composite surface containing bioactive glass and/or triterpenoids in a various method. In the present study, all types of experimental BAG containing composite reins (BAG, UA BAG, BAG + UA Monomer, UA BAG + UA Monomer) significantly reduced the amount of biofilm formation of *S. mutans* regardless of the salivary treatment and nutrient sources. When glucose was given as a carbohydrate source, treatment with ursolic acid show additional effects of decreasing biofilm formation. Especially among them, BAG + UA Monomer groups showed the lowest biofilm formation when glucose was given as nutrient. On the contrary, when sucrose was given as a carbohydrate source, there were no significant differences among BAG, UA BAG, BAG + UA Monomer, and UA BAG + UA Monomer groups.

The mechanism of antibacterial effect of the BAG may be fluoride-releasing capacity. This capacity can reduce biofilm formation. In addition, BAG powders shows the complete inhibition of the growth of *Streptococcus mutans* after only 2 hours of contact with the BAGs powder(Catauro et al., 2004). Furthermore, these materials are relatively inexpensive and are easy to synthesize (Mitchell et al., 2011).

Among various antibacterial agents, ursolic acid was selected for several reasons. First, ursolic acid has been shown to inhibit glucosyltransferase from *S.mutans*, a primary cariogenic bacteria(Kozai et al., 1987). Second, ursolic acid exhibited inhibition of *S.mutans* at a very low concentration (MIC_{90} = 2ug/ml) in

a planktonic condition(Kim MJ, 2011). Third, Hydrophobic nature of ursolic acids might induce them to blend properly with the composite resins and not easily eluted into the saliva, thus long-lasting antibacterial effects could be expected. In addition, ursolic acid is relatively non-toxic and had no side effect(Liu, 1995). Therefore, ursolic acid was also assumed to be very useful supplements of antibacterial composite resins. In this present study, the addition of ursolic acid was expected to decrease biofilm formation than BAG groups because ursolic acid-added groups showed stronger antibacterial effects than pure BAG group.

In the present study, when glucose was given as a carbohydrate source, the lowest biofilm formation of *S. mutans* on the ursolic acid was added to the resin matrix groups (BAG + UA Monomer groups). In case of addition to the resin matrix, significant additional antibacterial effect was seen than addition to the filler. It can be explained that when ursolic acid was incorporated into the resin matrix (BAG + UA Monomer groups), ursolic acid can be existed within the composite resin evenly compared to add into filler components. Also, in case of coating on the surface of BAG filler, ursolic acid could be detached from filler by polishing procedure and entrapped into polymer matrix during polymerization. Therefore antibacterial effect of ursolic acid would be decreased (Mitchell et al., 2011). In similar, our previous study showed significant antibacterial effects when the same concentration of ursolic acid (0.5%) was incorporated into the matrix of a commercial nanofilled composite (Kim, 2011). Also, other reports, which showed complete inhibition of bacterial growth, added antibacterial components into the resin matrix (Beyth et al., 2006).

However, the site of incorporation of antibacterial materials is controversial. Ebi et al reported that antibacterial materials are into filler components appears a more reliable approach than the addition into resin matrix. Because former option could allow increased amounts of antibacterial agents due to a larger surface area of micro or nanofillers (Ebi et al., 2001). Therefore, further studies are needed for determining the appropriate insertion site of antibacterial agents (resin matrix or filler components).

Generally, saliva coating could influence the maturation of biofilm (Ahn et al., 2008; Pecharki et al., 2005). But, saliva coating, depending on the carbohydrate source, the effect is different. In the present study, when glucose was given, significantly lower CFU values in the saliva coating group were found. Salivary coating decrease the surface free energy(Quirynen and Bollen, 1995) and block the affinity sites of bacterial adhesion(Yamaguchi, 2004).

On the contrary, when sucrose was given, significantly increased CFU values in the saliva coating group were seen. These results may be related to glucan formation. *S.mutans* could produce water-insoluble glucans when sucrose was given as nutrient. The glucans is the most important virulence factors of *S. mutans* and could promote adhesion and biofilm formation. Thus inhibitory effects of salivary coating on biofilm maturation may be neutralized when sucrose was given as nutrient(Cross et al., 2007).

The bacterial growth curve results did not show any difference among all groups contrary to those of biofilm assay. In all types of experimental composite resins, the bacteria entered stationary phase at an optical density in

both TV-glucose and TV-sucrose medium. First possible reason for the difference between biofilm assay and growth inhibition test is the mechanism of antibacterial effect of experimental resin. Biofilm assay is essentially based on measuring of direct and close contact between the test microorganism and the surface of test materials. On the other hand, growth inhibition test was developed to measure antibacterial effect of leaching-out of antibacterial components. Antibacterial effect of ursolic acid occurred at the interface between materials and bacteria by direct contact and ursolic acid cannot be leach-out surrounding area. Therefore the growth inhibition test results did not show antibacterial effect. The mechanism of antibacterial effect varies depending on the materials, the various antibacterial tests are needed. Second possible reason is the measurement method. Biofilm assay measured total cell number directly, but growth inhibition test measured cell mass by monitoring of optical density. Therefore, changes in optical density may not appear if there is not enough change in cell mass amount.

There are several limitations in present study. First, this study was performed only with *S.mutans*. Although *S. mutans* is one of the most important bacteria in the pathogenesis of dental caries and is involved in the initial process of dental caries, complex interactions between many species of other cariogenic bacteria influence the formation of biofilms and etiology of dental caries. Therefore, further studies should be performed with other cariogenic bacteria. Second, this study evaluated antibacterial effect of experimental composite resin in any salivary treatment and carbohydrate sources for less than 24 hours. Therefore, long-term study is needed.

V. Conclusion

Experimental composites containing BAG showed a significant reduction of the biofilm formation by S. *mutans* in any salivary treatment and carbohydrate sources for less than 24 hours. When glucose was given as a carbohydrate source, treatment with ursolic acid show additional effects of decreasing biofilm formation. Especially among them, composites containing BAG fillers and UA in the matrix group showed more significant antibacterial effect compare to other groups. On the contrary, when sucrose was given, treatment with ursolic acid did not show any additional effects of decreasing biofilm formation.

The bacterial growth curve results did not show any difference among all groups contrary to those of biofilm assay.

Within the limitation of this experiment, this result can be used for the development of the antibacterial composite in the future.

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국문 요약

Bioactive glass 와 ursolic acid 를 함유한 복합레진의 *Streptococcus mutans* 바이오 필름에 대한 항균효과

연세대학교 대학원 치의학과

(지도 교수 박정원)

김보나

1. 목적

졸-겔 생활성(生活性) 유리와 트리테르페노이드를 다양한 방법으로 첨가한 항균 복합 레진의 대표적인 치아 우식 유발 균인 Streptococcus mutans 에 대한 항균 효과에 대해 알아보고자 한다.

2. 재료 및 방법

충진재로 졸-겔 생활성 유리를 첨가한 복합레진 4가지를 제작하였다. 생활성 유리군과 생활성 유리 충진재에 우르솔릭산을 첨가한 군, 레진 기질에 우르솔릭산을 첨가한 군, 그리고 생활성 유리 충진재와 레진 기질 양쪽 모두에 우르솔릭산을 첨가한 군을 실험 군으로 하여 대조 군의 일반 복합레진과 비교하였다. 복합 레진 시편을 제작하고, 항균 작용을 평가하는데 있어 바이오필름 평가와 성장억제평가를

시행하였다. 바이오필름 평가를 위해 시편을 여과한 비자극 타액 또는 PBS(phosphate buffered saline) 로 코팅 후 포도당 또는 자당 배지를 사용하였다. S.mutans 를 복합레진 시편 상에서 24시간 배양 후 세균 배양된 시편을 음파처리하여 시편으로부터 세균을 분리한 후 집락형성 단위를 측정하여 평가하였다. 성장억제평가는 초승달 모양의 레진 시편을 well cluster 에 부착한 후 여과한 비자극 타액 또는 PBS 로 코팅 후 포도당 또는 자당배지를 사용하였고, TV media 를 넣고 흡광도 변화를 24시간 동안 시간에 따라 측정하여 세균 성장 여부를 관찰하였다. 통계처리는 Two-way ANOVA 및 Bonferroni 검정을 사용하였다.

3. 결과

바이오 필름 평가 시에는 생활성 유리를 첨가한 복합레진군이 (BAG, UA BAG, BAG + UA Monomer, UA BAG + UA Monomer) 대조 군에 비해 유의할 정도로 낮은 집락형성단위를 보였다. 포도당 배지에서 우르솔릭산을 첨가한 그룹이 부가적으로 유의할 정도로 낮은 집락형성 단위를 보였고, 그 중에서도 우르솔릭산을 레진 기질에 첨가한 그룹(BAG + UA monomer)이 다른 그룹에 비해 유의할 정도로 낮은 집락형성 단위를 보였다. 자당 배지에서는 우르솔릭산을 첨가한 그룹간에 유의차는 나타나지 않았다.

성장억제평가에서는 바이오필름 평가 시와 달리 항균작용이 관찰되지 않았다.

4. 결론

졸-겔 생활성 유리를 함유한 복합레진에서 최소한 24시간 이내에서는 치아 우식유발 균인 S.mutans 바이오필름에 대한 항균효과를 보였다. 포도당 배지에서 우르솔릭산 첨가에 의한 부가적인 항균작용이 나타났으며, 그 중 레진 기질에 우르솔릭산을 첨가한 군에서 항균효과가 가장 높았다. 자당 배지에서는 우르솔릭산의

첨가에 의해 부가적인 항균작용을 나타나지 않았다. 성장억제평가에서는 바이오필름 평가 시와 달리 항균작용이 관찰되지 않았다.

Key words : 생활성 유리, 우르솔릭산, 항균 복합레진, 바이오필름, *Streptococcus mutans*